Astrocyte inactivation of the pRb pathway predisposes mice to malignant astrocytome development that is accelerated by PTEN mutation

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Astrocytomomas

Little is known about the developing mechanisms

- Mutations or Changes
  - in the pRB pathway (70-80%)
  - in PTEN (50%)
    - in the AKT pathway

→ Experimental animal models are required
Question

Which pathways are involved in the development of astrocytoma?
The Rb pathway

[Diagram showing the Rb pathway with molecular interactions and regulatory processes involving INK4A, CDK4, CDK6, Cyclin D, RB, E2F, Cyclin E, CDK2, G1-S phase, transcription of S-phase genes, and differentiation apoptosis.]
The PTEN influence on the AKT pathway
What is there to be tested?

The pRb function is impaired in human astrocytomas at a high frequency.

- Test the impact of inactivating this pathway specifically in astrocytes of genetically engineered mice

- Do astrocytes respond similarly to Rbf inactivation?
- Does a defect in this pathway predispose to astrocytoma?
- Do mutations on p53 or PTEN cooperate with pRbf inactivation in astrocytoma development
Methods

- T121 was expressed transgenically in mice and used to dominantly inactivate pRb, p107, p130 in astrocytes.
- Transgene Design
  - A: GFAP-T121 transgene
  - B: GZT121 transgene
- TgGFAPT121 mice
  - Died perinatally
- TgGZT121 mice
  - Do not express T121,
  - 17 month
- TgG(ΔZ)T121 mice
  - Offspring of TgGZT121 crossed with Tgβ-actinCre
  - Became ill after 5-8 month
Results I

Astrocyte specific pRbf inactivation causes widespread brain abnormalities

- of 13 TgGFAPT\textsubscript{121} 10 developed severe brain abnormalities and died 7-9 days after birth
  - Abnormalities: hypercellular two distinct abnormal cell populations

![Image of normal and abnormal brain regions](image-url)
Results I

Diffuse cells (region II)
Express GFAP and T₁₂₁

Periventricular cells (region I)

Colocalisation of GFAP and T₁₂₁ in most T₁₂₁-expressing cells
Results II

Rb\(_f\) inactivation induces astrocyte proliferation and apoptosis

Proliferation

TgGFAP-T\(_{121}\) mice

Proliferation rates were determined for T\(_{121}\) expressing cells in regions of the brain that harbored anaplastic cells.
Apoptosis

Apoptosis in regions containing anaplastic astrocytes (E) or periventricular neural precursors (F)

Apoptotic cells relative to total cells in the examined regions
Results III

Inducible astrocyte Rb\(_f\) inactivation predisposes adult mice to high grade astrocytoma

Normal (TgGZT\(_{121}\))   Abnormal (TgG(ΔZ)T\(_{121}\))

Widespread infiltrating anaplastic cells

Brains with malignancies show widespread GFAP positivity

Anaplastic cells are also positive for S100 antigen
Results IV

Akt Kinase is regionally activated during astrocytoma development

- Long latency between $T_{121}$ expression (p7) and development of astrocytomas (>100 days)
  - Additional events to Rb\(_f\) inactivation

- Aktivation of Akt-Kinase by phosphorylation is positively regulated by PI3 kinase and PDK1 and negatively regulated by PTEN

- Akt activation protects cells from apoptotic stimuli
  - Apoptosis levels were greatly reduced in Akt active regions
  - Akt activation may provide a selective advantage to astrocytoma cells initiated by pRb\(_f\) inactivation by reducing apoptosis
## Results IV

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<thead>
<tr>
<th>Activated Akt</th>
<th>T121</th>
<th>Apoptosis</th>
<th>PCNA</th>
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<tr>
<td>A</td>
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<td>E</td>
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adjacent region
PTEN, but not p53, heterozygosity, accelerates astrocytoma tumorigenes

- Inactivating mutations of PTEN occur frequently in high grade human astrocytoma (50%)
- Mutations of p53 occur at much lower frequency (30%) and often appear at early stages

→ Effect of both p53 and PTEN heterozygosity on \( T_{121} \) induced astrocytoma development
Results V

TgG(ΔZ)T\textsubscript{121} mice heterozygous for null alleles of either gene

→ p53 haplodeficiency did not reduce the latency of astrocytoma
→ PTEN+/− developed enlarged crania
  → not clear if haploinsufficiency or PTEN inactivation is required for tumor acceleration
→ PTEN mutation contributes to astrocytoma development

\[ \Delta = \text{PTEN +/− mice} \]
\[ \square = \text{p53+/− mice} \]
\[ \diamond = \text{TgG(ΔZ)T}_{121} \text{ mice} \]
\[ \bullet = \text{TgGZT}_{121} \text{ mice} \]
Summary

- Inactivation of the pRb pathway in astrocytes induces aberrant proliferation and extensive apoptosis and can constitute an initiating event in the development of high grade astrocytomas.
  - Progresses to malignant anaplastic astrocytomas in adult mice.
  - 70% show areas of decreased apoptosis and express activated Akt.
  - PTEN mutation contributes to astrocytoma development.
Summary

- TgG(ΔZ)T\textsubscript{121} astrocytoma model resembles the human disease with respect to both the molecular targets and the ensuing pathology
  - Useful for further mechanistic elucidation and possibly for preclinical therapeutic testing
- TgGZT\textsubscript{121} mice in which pRb inactivation can be induced somatically, will provide an excellent model for exploring aspects of tumor evolution
- But: mice may die of expansive astrocytoma before significant tumor progression occurs
Articles

- Astrocyte inactivation of the pRb pathway predisposes mice to malignant astrocytoma development that is accelerated by PTEN mutation

- Tumour-suppressor functions in the nervous system